PCR-terminal restriction fragment length polymorphism for direct detection and identification of dermatophytes in veterinary mycology

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Résumé en anglais	The biological diagnosis of dermatophytosis in veterinary medicine usually relies on direct microscopic examination and inoculation of the samples on appropriate culture media. However, identification of dermatophytes needs expertise, and cultures which require from days to weeks to be conclusive, may lack of sensitivity because of the quite common overgrowth of contaminants. Here we developed a polymerase chain reaction (PCR) assay based on terminal restriction fragment length polymorphism (TRFLP), which may improve sensitivity of the biological diagnosis and reduce the delay for initiation of treatment. This study was first conducted on pure cultures of various dermatophytes (27 species), yeasts (14 species) and moulds (45 species). After DNA extraction, the internal transcribed spacer (ITS)-28S region of ribosomal DNA was amplified with primers targeting specifically pathogenic dermatophytes, and species of interest were identified by TRFLP with appropriate restriction enzymes. After validation, this assay was applied to veterinary samples and results were compared to those obtained by direct microscopic examination and cultures. All target species were correctly identified, and none of the yeast or mould species was amplified, demonstrating specificity of the assay. Regarding clinical samples, the causative agent was detected by PCR-TRFLP from 97.1% of the samples with both positive direct microscopic examination and cultures. No dermatophytes were detected when both conventional tests were negative. PCR-TRFLP developed here demonstrated to be highly sensitive and specific, allowing rapid detection and direct identification of dermatophytes in veterinary practice. Therefore, this assay is especially suitable for the biological diagnosis of dermatophytosis in different animal species.
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